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Three new records of plant parasitic phyllosphere fungi from Panama: *Annellophora phoenicis*, *Cercospora corniculatae*, and *Sclerotium coffeicola*

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Abstract

Three fungi associated with living leaves of plants are new records for Panama: *Annellophora phoenicis* causing leaf spots of *Cocos nucifera* (Arecaceae), *Cercospora corniculatae* (*C. apii* s. lat.) on living leaves of *Oxalis barrelieri* (Oxalidaceae) with and without discoloration, and *Sclerotium coffeicola* on zonate leaf spots of *Annona montana* (Annonaceae) and *Dioscorea alata* (Dioscoreaceae). Some records of *A. phoenicis* and *S. coffeicola* relevant for known geographical distribution and available by literature are critically revised.

Key words

Anamorphic fungi; asexual fungi; Ascomycota; Basidiomycota; tropical fungi.

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Introduction

Fruit production plays an important role in the economy of Panama. Bananas and coffee belong to the most important products for export. Although plant protection agencies need well-documented data about plant pathogenic fungi, scientifically presented information about pathogens of cultivated plants is far from being complete for Panama. Inventories of plant parasitic fungi, therefore, even recently, reveal new records for the economically important fruit plants, such as *Asterinella puiggarii* on guava (Piepenbring et al. 2011) and *Ramichloridium biverticillatum* and *R. musae* on banana (Kirschner and Piepenbring 2014).

Methods

Specimens were collected during July 2016 in Chiriquí Province, at the border of a small village in the lowland of western Panama, approx. 150 m above sea level (a.s.l.), with authorization from the Ministerio de Ambiente (MiAmbiente, Panama). Colors of symptoms on leaves were observed using fresh material. Specimens of *Sclerotium coffeicola* were loaned from the Fungarium Collections of the Herbarium of Royal Botanic Gardens, Kew (K). Fungal material was mounted in 10% KOH, with and without previous staining with aqueous Kongo red solution, or in cotton blue in lactic acid and polyvinyl alcohol. Statistical treatments of the measurements are

given with extreme values in brackets and mean value ± 1 standard deviation of *n* measurements. Specimens were air-dried on an electrical dryer and deposited at the Herbario de la Universidad Autónoma de Chiriquí, Panama (UCH). A sequence (603 bp) of the internal transcribed spacer (ITS) of the ribosomal RNA gene (rDNA) from a dried specimen of *S. coffeicola* was generated as described in Kirschner (2016), submitted to a BLAST search and deposited in GenBank (MF170962).

Results

Ascomycota, incertae sedis

Annellophora phoenicis M.B. Ellis, Mycol. Pap. 70: 87 (1958)
Figure 1

Leaf spots amphigenous along the leaf margins, reddish brown, with bright yellow margin, 10–35 × 3–7 mm, becoming confluent. Stromata lacking. Hyphae penetrating into and through cell walls of the epidermis, forming small empty lacunae and cyanophilous areas within the outer epidermis cell wall, intracellular hyphae inconspicuous and rare in the epidermis, conspicuous in the mesophyll cells, pale brown, smooth, 1–3 μm wide. At the colony margin, hyphae are yellowish pale brown, smooth, occasionally verruculose, 1–2 μm wide, loosely ramified and without appressoria; in the older part, the hyphae are densely ramified with numerous swollen intercalary appressoria showing a central round pale spot, 4–7 × 2.5–4 μm. Conidiophores predominantly epiphyllous, arising solitarily from a lobed foot cell (lobes only visible in young conidiophores), being 10–15 wide and 4–5 μm high, erect, straight, unbranched, cylindrical, brown, smooth, two- to several-celled, with some intercalary cells darker than the other cells, length including foot cell (10–)25–50(–65), width 4(–5) μm (*n* = 30). Conidiogenous cells terminal, with percurrent extensions, truncate at the apex, 4–11.5(–15) × 3–4 μm (*n* = 30). Conidia broadly clavate or obclavate, sometimes with 1–2 constrictions, yellowish pale brown, smooth, distoseptate in the main body, rarely forming percurrent extensions of the transversally 0–12-septate beak or a secondary conidium at the end of the beak, septa per conidium altogether (9–)10–14(–16) (*n* = 30), distances between septa 3–6 μm, size including beak (45–)56–75(–86) × (10–)10.5–12.5(–13) μm (*n* = 30), hilum 3–4 μm wide.

Specimen examined/new record. Panama, Chiriquí province, Corr. Dolega, Los Algarrobos, Casa de la Alemana, garden, approx. 150 m a.s.l. (08°29′45″ N, 082°25′58″ W), on living leaves of *Cocos nucifera* L. (Arecaceae/Palmae), 23 July 2016, R. Kirschner 4292 (UCH).

Known distribution (Fig. 2). "Malaya" = West Malaysia (Malaysia), Myanmar, New Guinea (Indonesia/Papua New Guinea), Sierra Leone (Ellis 1971, Thaung 2008), India? (Shanthi and Vittal 2012), Thailand? (Kodsueb et al. 2008), Panama (new record).

Notes. The species is identified based on the almost identical morphology described by Ellis (1958) and its occurrence on palms. This fungus has been recorded from *Cocos* and *Phoenix* species in the Old World (Ellis 1971). In contrast to Ellis (1958, 1971), we rarely found percurrent extensions of the conidium beak and we observed inconspicuous, intercalary appressoria among superficial hyphal cells. A more detailed study, however, is required to reveal the exact mode of penetration into the leaf. In our opinion, a report as causative of palm leaf spot from Texas by Vann and Taber (1985), frequently cited in American textbooks (Horst 2001, Elliot et al. 2004, Broschat et al. 2014), is based on a wrongly identified specimen, because the distinct sporodochia formed by stout conidiophores and the conspicuously verrucose conidia shown in the scanning electron microscopic photograph, as well as gradual tapering of conidia from the base to the end instead of having an abrupt beak shown in the light microscopic photograph, clearly indicate that the agent of palm leaf spot from Texas should be called *Scolecostigmina palmivora* (Sacc.) Kamal. Ellis (1958) described the type specimen of *A. phoenicis* from dead palm leaves so that it is not clear whether the fungus was saprobic or parasitic. Reports from dead wood of *Michelia baillonii* (Magnoliaceae) in Thailand (Kodsueb et al. 2008) as well as from leaf litter of *Anacardium occidentale* (Anacardiaceae) in India (Shanthi & Vittal 2012) without any data on morphology may refer to other species. The considerably larger sizes of *A. phoenicis* var. *cubensis* Hol.-Jech. described from a dead fallen branch of an undetermined tree in Cuba (Holubová-Jechová 1988) would justify raising the variety to species level. The present concept of *Annellophora*, however, of being *Sporidesmium*-like but forming secondary conidia from percurrently extending conidial apices (Ellis 1958, 1971), appears artificial, because it comprises species from living leaves, dead wood, as well as other fungi (Ellis 1971). Since more recent reliable records of *A. phoenicis* after Ellis (1971) and molecular sequence data are not available, detailed microscopic documentation of similar fungi associated with palm leaf spots is necessary in order to approach correct species characterization.

Ascomycota, Capnodiales

Cercospora corniculatae Hansf., Proc. Linn. Soc. London 155: 56 (1943) [1942–43], (= *C. apii* s. lat., Crous and Braun 2003)
Figure 3

Leaf spots absent, sporulation taking place on green or diffusely reddish or yellow discolored leaves. Stromata absent or reduced to few cells. Hyphae internal, pale brown to hyaline, smooth, 2–4 μm wide. Conidiophores amphigenous, penetrating through stomata, solitary or in fascicles of up to 15, unbranched, rarely with one branching at the base, cylindrical, geniculate, brown, smooth, 2–7-septate, septa 5–25 μm apart, (65–)86–157(–205) × (5–)5.5–6.5(–7.5) μm (*n* = 30). Conidiogenous cells

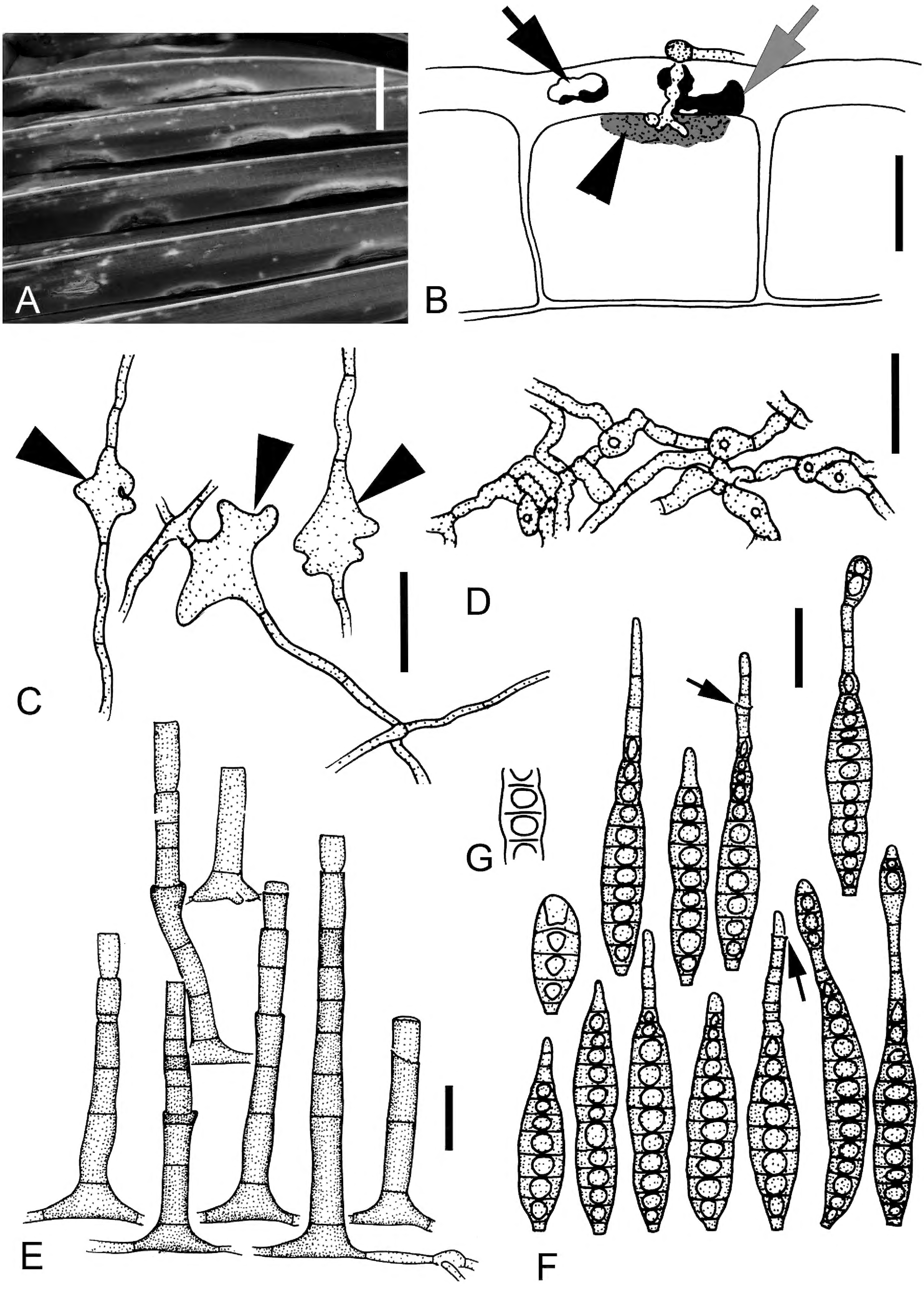

Figure 1. *Annellophora phoenicis* on *Cocos nucifera*. **A.** Leaf spots. **B.** Hypha that penetrated from appressorium into epidermis wall and cell. Lacuna in cell wall indicated with black arrow, cyanophilous cell wall reaction indicated with gray arrow, matrix with black arrowhead. **C.** External hyphae from colony margin with conidiophore bases (arrowheads). **D**. External hyphae from older part of colony with circles indicating penetration sites. **E.** Conidiophores. **F.** Conidia. Percurrent extension of two conidia is indicated by arrows. The three most right ones apically develop a secondary conidium. **G.** Detail of a conidium showing distosepta, pigmentation not shown. Scale bars: A = 1.5 cm, B = 5 µm, C–G = 10 µm.

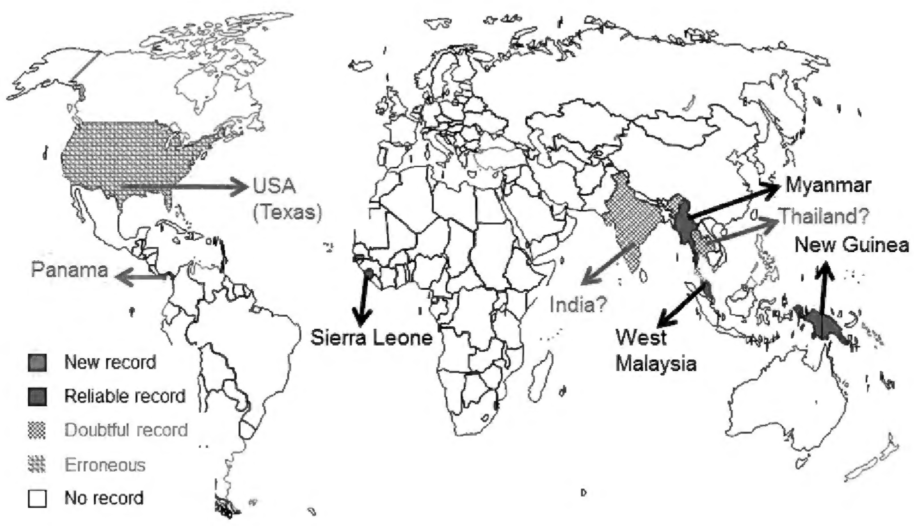

Figure 2. *Annellophora phoenicis*. World distribution, countries reliably reported in the literature marked with green, Panama (new record) with brown red. Blue color indicates records considered erroneous or doubtful in this study.

intercalary and terminal, straight or geniculate, pale brown to brown, smooth, terminal ones (22–)31–50(–56) × 4–6(–7) μm (n = 30). Conidiogenous loci conspicuous, darkened, 1 μm thick, 2–3 μm wide. Conidia solitary, obclavate-cylindrical, straight or slightly undulate, sometimes the distal part curved, hyaline, smooth, 2–11-septate, (21–)51–100(–130) × 4–5 μm (n = 30), basal hilum thickened, darkened, 2–3 μm wide.

Specimen examined/new record: Panama, Chiriquí province, Corr. Dolega, Los Algarrobos, approx. 150 m a.s.l. (08°29′45″ N, 082°25′58″ W), on living leaves of *Oxalis barrelieri* L. (Oxalidaceae), 25 July 2016, R. Kirschner 4293 (UCH).

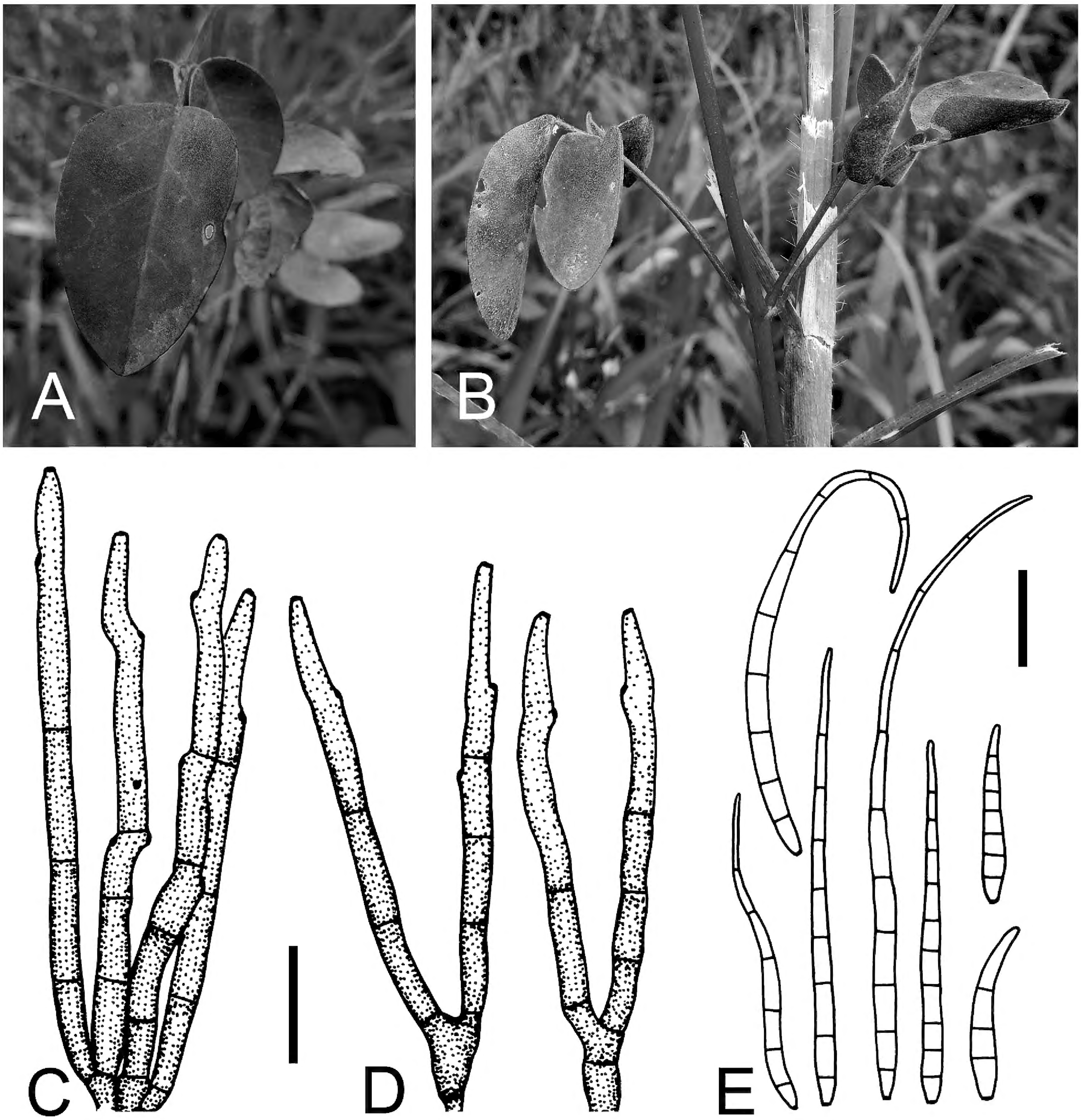

Figure 3. *Cercospora corniculatae* (*C. apii* s. lat.) on *Oxalis barrelieri*. **A.** Upper leaf surface covered by brown fascicles of conidiophores. **B.** Upper and lower leaf surfaces covered by brown conidiophores fascicles and white conidial mass. **C.** Fascicle of four conidiophores. **D.** Two of the rare basally branched conidiophores. **E.** Conidia. Scale bars = 20 μm.

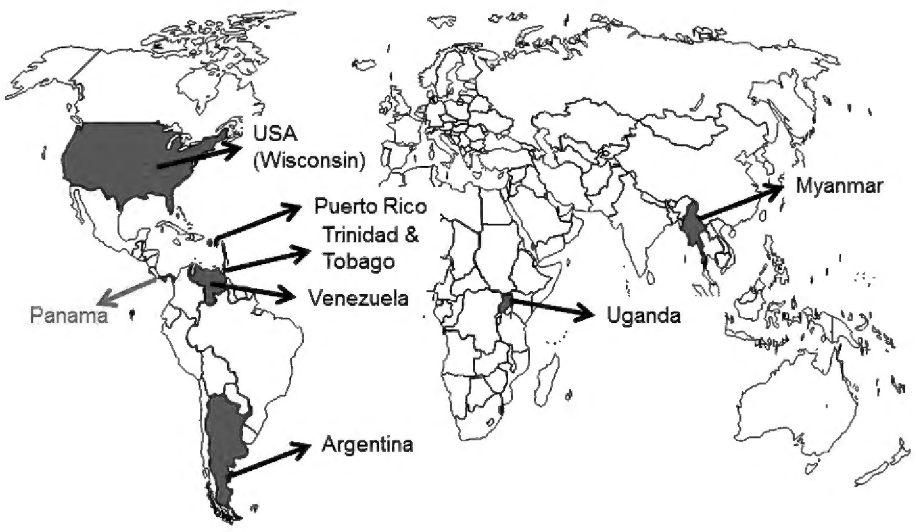

Figure 4. *Cercospora corniculatae* (*C. apii* s. lat.). World distribution, countries reported in the literature marked with green, Panama (new record) with brown red.

Known distribution (Fig. 4). Argentina, Myanmar, Puerto Rico, Trinidad and Tobago, Venezuela, Uganda, USA (WI) (Crous and Braun 2003, Farr and Rossman 2017), Panama (new record).

Notes. This is the first record of a *Cercospora* species on members of *Oxalis* in Panama. The other known *Cercospora* species on members of *Oxalis*, *C. oxalidis* A.S. Mull. & Chupp ex U. Braun & Crous, differs by its overall smaller dimensions from *C. corniculatae/C. apii* (Crous and Braun 2003). *Cercospora corniculatae* has been recorded from *O. barrelieri* in Trinidad and Tobago and Venezuela (Farr and Rossman 2017). On other *Oxalis* species, the fungus was also recorded from further countries (Crous and Braun 2003). In contrast to the descriptions of *Cercospora* spp. on *Oxalis* spp. (Chupp 1954, To-Anun et al. 2011), distinct leaf spots were not present in the Panamanian specimen, but conspicuous sporulation took place on non-discolored green as well as diffusely discolored leaves. The type of the invalid *C. oxalidiphila* Chupp & A.S. Mull. was studied by Braun (2000), who indicated the synonymy with *C. corniculatae* and suggested a close relationship or identity with *C. apii*. Species delimitation in the *Cercospora apii* species complex (Crous and Braun 2003) is still under progress (Groenewald et al. 2013).

Basidiomycota, Atheliales

Sclerotium coffeicola Stahel, Bull. Dep. Landbouw Suriname 42: 15 (1921)
Figures 5, 6

Leaf spots zonate by alternating concentric pale and dark brown rings, with dark brown margin, 8–95 × 8–55 mm. Propagules hypophyllous, white, erect, lanceolate, smooth, 2–4 × 0.1–0.2 mm, easily detaching from the leaf. Propagules composed of textura intricata at the base, over the length composed of parallel; scarcely branched, hyaline, smooth or verruculose hyphae of 4–6 µm diameter; septa 20–70 µm apart, converging at the tip.

Specimen examined/new record. Panama, David, Corr. Dolega, Los Algarrobos, Casa de la Alemana, garden, approx. 150 m a.s.l. (08°29′45″ N, 082°25′58″ W), on living leaves of *Annona montana* Macfad. (Annonaceae), 25 July 2016, R. Kirschner 4290 (UCH; ITS sequence: GenBank MF170962).

Additional specimens examined. Sierra Leone, Njala (Kori), on leaves of *Jasminum multiflorum* (Burm. f.) Andrews [as *J. pubescens* (Retz.) Willd.], 1933, F.C. Deighton M468, IMI 35109 (K, only sclerotia); same

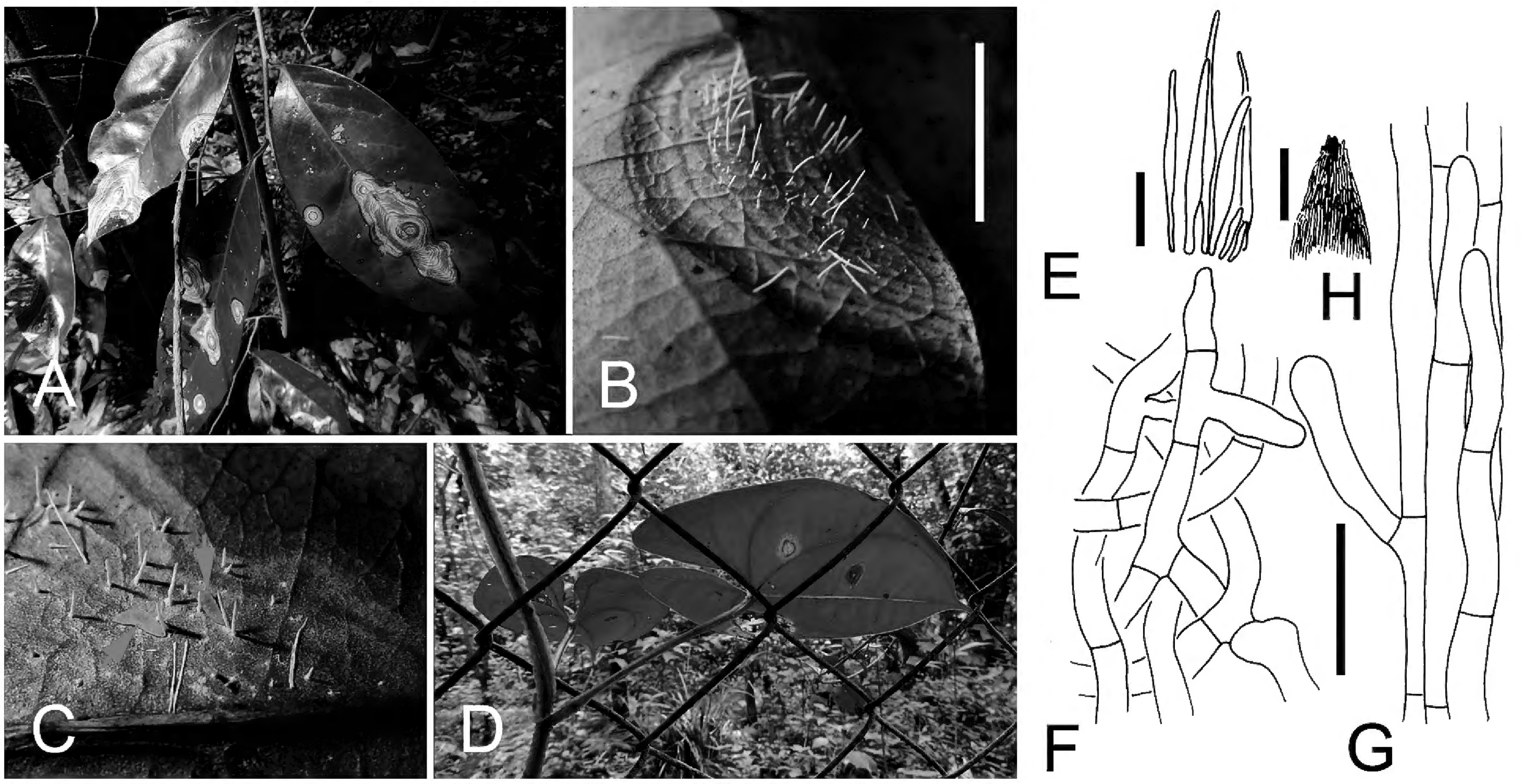

Figure 5. *Sclerotium coffeicola* from Panama, fresh material on *Annona montana*, except D: on *Dioscorea alata*. **A.** Concentric zonate spots seen on the upper leaf side. **B.** Leaf spot seen from below showing the white lanceolate propagules. **C.** Lower leaf side showing propagules and orange gall midge larvae (blue arrows). **D.** Abaxial leaf spots of *Dioscorea alata*. **E.** Habitus sketch of propagules. **F.** Detail from the textura intricata of the base of the propagule. **G.** Detail from the parallel hyphae composing the main body of the propagule. **H.** Apex of propagule showing convergent hyphae. Scale bars: B = 1 cm, E = 1 mm, I = 100 µm, F, G = 20 µm.

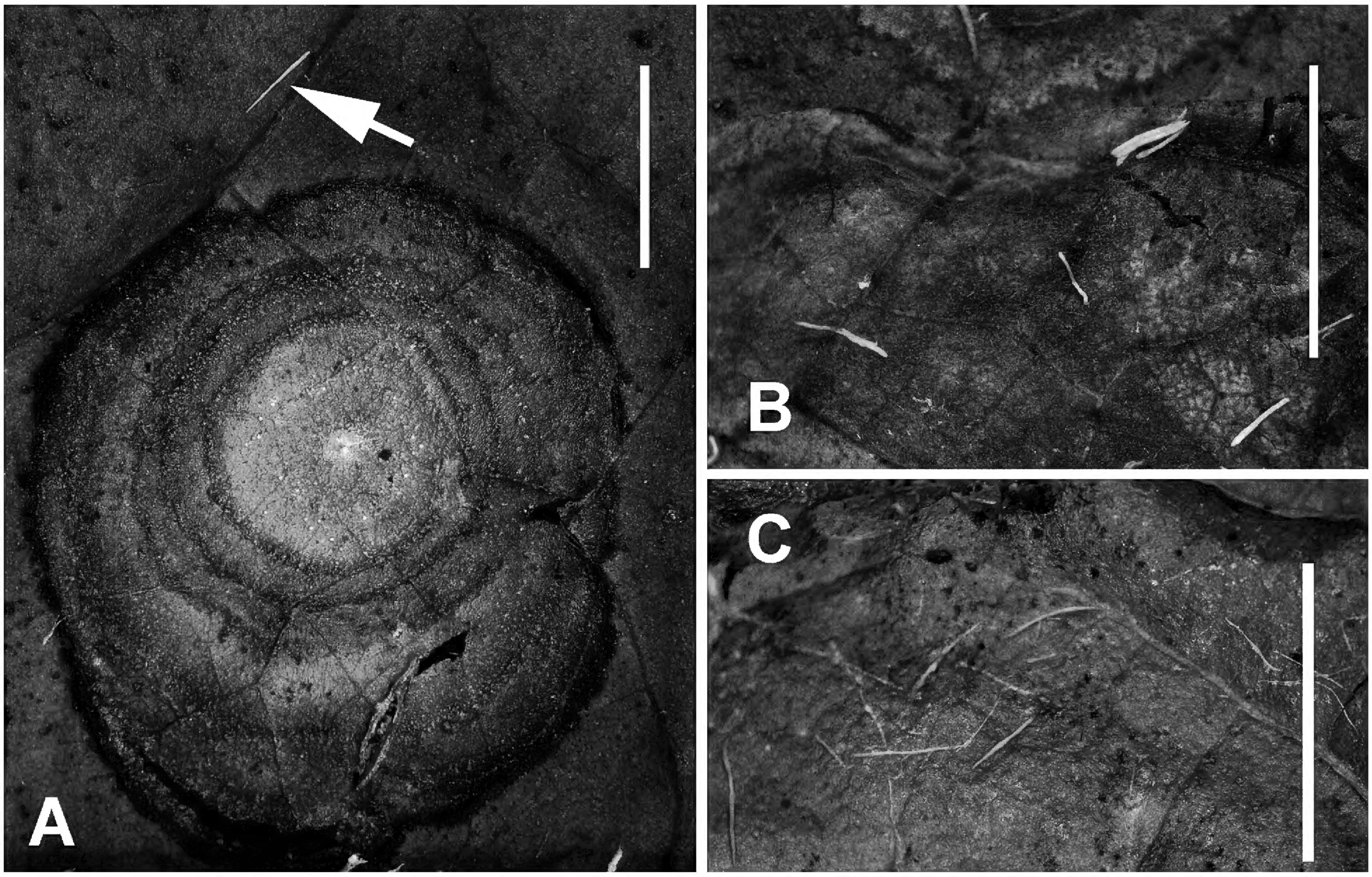

Figure 6. *Sclerotium coffeicola* from Africa (Sierra Leone), collections from 1949 (K). **A.** Concentric leaf spot on the upper side of leaf of *Aristolochia inflata* (IMI 37953), a single propagule marked with arrow. **B.** Propagules from lower side of leaf of *A. inflata* (IMI 37953). **C.** Propagules from abaxial leaf side of *Jasminum multiflorum* (IMI 37954). Scale bars = 4 mm.

area, on zonate leaf spots of *Aristolochia inflata* Kunth (as *A. gibbosa* Duch.), August 1949, F.C. Deighton M2944, IMI 37953 (K); same area, on leaves of *J. multiflorum* (as *J. pubescens*), August 1949, F.C. Deighton M3030, IMI 37954 (K).

Known distribution (Fig. 7). Brazil, Costa Rica, Guyana, Puerto Rico, Suriname, Trinidad and Tobago, Venezuela (Hanlin and Tortolero 1989), Panama (new record), Sierra Leone (Deighton 1936).

Notes. On leaf spots, only the lanceolate white propagules were observed, which agree to the description as "columnar bundles of hyphae" by Hanlin and Tortolero (1989), but mycelia or sclerotia also described by Hanlin and Tortolero (1989) were not found. The ITS sequence from the dried specimen from Panama had 99% similarity with that of the ex-type culture (CBS 115.19, GenBank NR_145331, Okabe and Matsumoto 2003) as well as with an unpublished sequence of *S. coffeicola* (GenBank KP176676), whereas the next similar sequences in the BLAST search were all from *Athelia rolfsii* (Curzi) C.C. Tu & Kimbr. with 93% similarity. In some leaf spots, the fungus was associated with orange colored larvae of gall midges (Cecidomyiidae) apparently feeding on it. The same fungus was also observed on *Dioscorea alata* (Dioscoreaceae, material not preserved) partially growing below and partially climbing into the infected *Annona* tree. On this liana, leaf spots and sporulation were sparse.

Sclerotium coffeicola is known from different host plant families and geographically limited to the neotropical region, except for Africa (Sierra Leone; Farr and Rossman 2017). Re-examination of records from the paleotropical area revealed to represent *S. rolfsii* Sacc. [= *Athelia rolfsii* (Curzi) C.C. Tu & Kimbr.] (Hanlin and Tortolero 1989), but did not include specimens from Sierra Leone deposited by F.C. Deighton in K. Our study of the specimen of *S. coffeicola* published by Deighton (1936) only revealed sclerotia so that we were unable to confirm the species identification. Additional specimens, however, from the same locality collected by Deighton in 1949, contained the lanceolate propagules, so that we could confirm the identification of the African specimens as *S. coffeicola*. Hanlin and Tortolero (1989) compiled

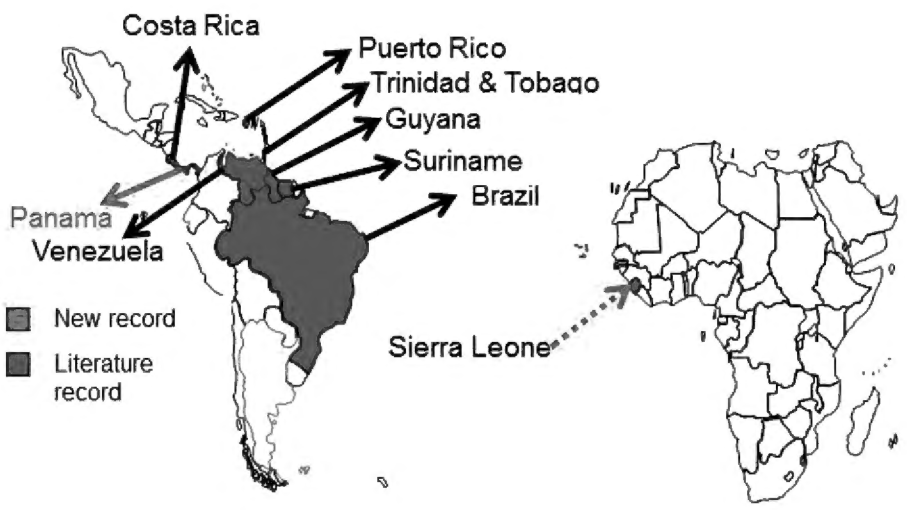

Figure 7. *Sclerotium coffeicola*. World distribution, countries reported in the literature marked with green, Panama (new record) with brown red, Sierra Leone (published record confirmed by re-examination) with green color and brown red dotted arrow.

the knowledge about the geographic distribution, hosts, and heavy impact on coffee plantations, and among other new information, provided descriptions of the sclerotium development and ultrastructure. The ultrastructure indicated the basidiomycetous relationship, although a teleomorph is not yet known. In spite of the importance of coffee plantations for the economy of Panama, publications about *S. coffeicola* on coffee leaves in Panama are not known to us, whereas *Mycena citricolor* (Berk. & M.A. Curtis) Sacc. is a well-known threat for coffee in Panama (Piepenbring 2006). The systematic placement of *S. coffeicola* in the Atheliaceae is also unclear, since in spite of the available rDNA sequences, a conclusive phylogenetic analysis has not been undertaken (Xu et al. 2010).

Discussion

Since knowledge about the fungal diversity in Panama is comparatively limited, basic research providing specimens in publicly accessible collections, and detailed descriptions and illustrations in international scientific journals as well as correctly annotated sequence data is required for decision-making by plant protection agencies (Piepenbring et al. 2011). Because of restrictive laws concerning export of living organisms, of lack of a public culture collection, and of limited laboratory facilities in Panama, however, cultures could not be preserved so that DNA methods could only be applied to dried material of the macroscopically visible propagules of *Sclerotium coffeicola*. For the other 2 new records from Panama, *Annellophora phoenicis* and *Cercospora corniculatae*, DNA sequences would be helpful in order to address the open questions of probably wrongly identified specimens of the former and the host range of the latter. Concerning the doubtful records of *A. phoenicis* in the literature, only the morphology of the specimen from the USA was presented in detail by Vann and Taber (1985), so its identification could be corrected as *Scolecostigmina palmivora*, without the necessity to trace and study the original material by ourselves. Since *A. phoenicis* belongs to quarantine organisms in some countries, e.g. Egypt (Mohamed Ibrahim Ahmed 2010), exact species identification is important. Our re-examination of unpublished specimens of *S. coffeicola* confirmed the previous published record from Africa and again highlights the scientific importance of curating duplicates of unpublished specimens (Kirschner 2016). In *Cercospora*, the accumulation of results of infection experiments and DNA data showed that the concept of a host family-specific specificity does not work for several species so that numerous *Cercospora* species have been comprised under *C. apii* s. lat. (Crous and Braun 2003). Even in multigene studies, several species complexes could not be resolved, but some species appeared to be quite host specific, whereas others have broad host ranges among different plant families (Groenewald et al. 2013). The species identified here as *C. corniculatae/C. apii* may not be limited to *Oxalis* species, but spread to economically important plants from these weeds. The unclear taxonomy on the one hand and the potential phytopathological significance on the other make detailed documentations of preliminarily identifiable specimens particularly important as base for advanced studies.

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Authors' Contributions

MP and OC organized the infrastructure and its maintenance for collection and study of specimens, and identified host plants, OC arranged the collection permit, RK made the microscopy and identified the specimens, RK and MP did the literature research and wrote the text.

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